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R C van Dijk

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Identification of a novel liquid phosphate phosphatase prg-1 (plasticity related gene-1) involved in axonal outgrowth and regnerative sprouting

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Identification of a novel liquid phosphate phosphatase PRG-1 (plasticity related gene-1) involved in axonal outgrowth and regenerative sprouting

Description

Axons in the central nervous system (CNS) elongate through the extracellular space over long distances (*1*). This occurs during development (*2, 3*) and during axonal sprouting in response to partial deafferentation (*4, 5*). The extracellular space, however, is an outgrowth repellent environment that allows axonal elongation only under specific molecular conditions (*6*). Molecules involved in axonal outgrowth, such as semaphorins, netrins, or ephrins (*7, 8, 9, 10*), are able to transduce outgrowth promoting as well as inhibiting signals to elongating axons via specific receptors. Here, we provide evidence for a novel molecular mechanism by which axons are able to elongate in a phospholipid-rich environment that would normally inhibit outgrowth of fibers (*11, 12*). Specific upregulation of this novel member of the lipid phosphate phosphatase (LPP) family acting as an ecto-enzyme on axons allows local depletion of lipid phosphates, enabling fiber outgrowth through the repellent extracellular environment.

In the hippocampus, afferent connections from the entorhinal cortex enter in a layer-specific manner during development (*13*). This specific axonal navigation depends on molecular cues expressed along the pathway and in the target region (*13*). Transection of entorhinal axons in the adult leads to a specific deafferentation in the hippocampus with subsequent regenerative axon sprouting by remaining afferents into the denervated zones (*4, 14*). In an attempt to identify the molecular cues that govern this structural plasticity, we performed a differential cDNA screening approach for mRNAs specifically upregulated during development and in the lesioned hippocampus (*15*). We identified a new gene of 716 amino acids (aa) (Fig. 1A) which we named plasticity-related gene-1 (PRG-1; GenBank acc. #, submitted). This gene encodes a yet uncharacterized member of the LPP-1 membrane-associated phosphatic acid phosphatase ecto-enzyme family. These molecules have 6 membrane spanning domains with their active site located on the external surface of the plasma membrane. They have increasingly attracted interest because they are involved in modulating the specific signaling of bioactive lipid phosphates such as phosphatidate (PA), lysophosphatidate (LPA) or

sphingosine-1-phosphate (S-1-P) in the context of cell migration, mitogenesis, and neurite retraction (*16, 17, 18*). It has been shown that signaling via extracellular LPA plays an important role in CNS development and that postmitotic neurons are at least one endogenous source for LPA in the nervous system (*19*). Similar to other members of the LPP-family, hydrophobicity analysis of PRG-1 predicts six N-terminal membrane-spanning regions with highly conserved phosphatase domains. However, unlike any other member of this family, the second half of the protein consists of a long hydrophilic domain of around 400 aa (Fig. 1B). According to the structural models of LPP orientation in the membrane, this C-terminal extension is positioned on the cytoplasmatic site and might thus play a role as a regulatory or signal transduction domain. Besides the homology of the N-terminal part of PRG-1 to other members of the LPP-family such as LPP-1 and the Drosophila cell migration modulator Wunen, GenBank searches revealed only one other related gene (genomic DNA sequence: GenBank acc. # NP_079164), which we cloned and named PRG-2 (GenBank acc. #, submitted). This gene shares the same C-terminal extension with partial sequence homology, but shows a different expression pattern than PRG-1 (data not shown). Thus, these genes represent a novel distinct subclass of the LPP-1 family. Amino acid residues which have been shown to be essential for ecto-enzyme activity in the LPP-1 class of proteins are conserved in PRG-1 N-terminal sequences (Fig. 1A) (*20, 21*). Database analysis of the C-terminal domains did not detect any significant similarities to any other protein or any matches with known conserved domains (ProDom and Swiss-Prot databases). GenBank search for orthologous proteins show that both genes are highly conserved in mammals (human/mouse > 93%), and partial EST sequences indicate orthologous proteins in Xenopus and Zebrafish, whereas no significant homology for the C-terminal part could be found in the Drosophila or other invertebrate genome. Northern blot analysis revealed one distinct band and shows that PRG-1 mRNA expression is CNS-specific, with the exception of a weak expression in testis (Fig.

1C). Thus, PRG-1 appears to be a novel vertebrate specific protein, selectively located in the brain with putative phosphatase function.

In situ hybridization analysis highlighted tight regulation of PRG-1 transcripts in the developing hippocampus (*15, 22*). At embryonic day 16 (E16), no PRG-1 transcripts can be detected in the brain (Fig. 1D). An expression signal first appears at E19 in the subventricular zone and specifically in the hippocampal anlage, whereas other cortical regions do not show PRG-1 expression (Fig. 1D). From postnatal stages on, PRG-1 mRNA is present in the hippocampus and in the entorhinal cortex throughout adult stages (Fig. 1D). In the dentate gyrus, a region bearing postnatally developing granule cells (*23*), weak PRG-1 mRNA expression is found in the infrapyramidal blade at P0, whereas the later developing suprapyramidal blade first showed expression signals at P5. This expression pattern remains unchanged during maturation; however, a reduced expression is apparent in the adult brain.

We next analyzed PRG-1 mRNA expression following entorhinal cortex lesion which leads to a layer-specific denervation of the hippocampus followed by regenerative ingrowth of sprouting axons (*5, 14, 27*). PRG-1 is upregulated one day after lesion (dal) and peaks at 5 dal in the ipsilateral hippocampus (gcl = 37%, hilus 300%, CA1 = 100%, CA3 = 60%). The contralateral hippocampus (maximum by 1 dal, gcl = 16%, hilus = 200%, CA1 = 59%, CA3 = 46%), as well as the ipsilateral cortex, shows a strong upregulation of PRG-1 mRNA (maximum by 1dal 83%) (Fig. 1E).

Transfection studies using a PRG-1 construct tagged with the eGFP reporter gene reveals its processing in COS-7 cells through the Golgi apparatus (data not shown) to its final localization in the plasma membrane of small processes (Fig. 2A). To localize PRG-1 protein *in vivo*, we generated an antiserum against a peptide sequence from the cytoplasmic C-terminus of PRG-1 (*15*). This antiserum specifically stains transfected COS-7 cells expressing PRG-1-eGFP fusion protein (Fig. 2A). Both Western blot and immunostaining could be blocked by specific peptide incubation prior to the antiserum (data not shown).

Immuncytochemical staining of rat hippocampus reveals PRG-1 specifically in neurons (Fig. 2B) (*15*). Five days after entorhinal cortex lesion, a clear immunoreactive PRG-1 positive band appears in the denervated outer molecular layer (Fig. 2B), apparently representing single axonal processes which form terminal branches (Fig. 2B). To show that PRG-1 is indeed localized in regrowing axons following lesion, we performed an ultrastructural analysis. Immunoreactivity could indeed be localized to growth cone-like axonal structures in the denervated zones of the hippocampus (Fig. 2C).

Members of the LPP-family are known to dephosphorylate extracellular phospholipids such as lysophosphatidic acid (LPA). To study the phosphatase activity of PRG-1, we chose LPA as a simple phospholipid that has properties of an extracellular neurite repellent factor (*16, 29*). It is present in the extracellular space of the central nervous system (*19, 30*) and mediates diverse cellular responses through the activation of multiple signal transduction pathways (*17, 24*). One major structural effect of LPA on neurons is rapid neurite retraction with subsequent cell rounding. In order to provide evidence that PRG-1 is involved in axonal outgrowth during hippocampal development, we studied the effect of PRG-1 expression on LPA induced neurite retraction in living brain tissue. *In vivo*, axons start to grow from the entorhinal cortex towards the hippocampus at about E19 (*3*), which is parallel to the developmental onset of PRG-1 expression (Fig. 1D). Thus, we compared the response of entorhinal explants obtained before PRG-1 expression (at E16) to that of postnatal explants which express PRG-1 (*15*). Both embryonic and postnatal explants grow equally well under serum-free culture conditions and show long extending axons. However, their response to LPA differed dramatically (Fig. 3A). Whereas application of 10 μM LPA leads to rapid neurite retraction in embryonic entorhinal explants (E16; n = 32), postnatal explants (P0; n = 36) did not differ significantly from vehicle treated control cultures (Fig. 3B). These experiments indicate that postnatal entorhinal axons expressing PRG-1 are resistant to LPA-induced neurite retraction. To test if differential PRG-1 expression is directly responsible for these effects, we expressed PRG-1

protein in neuronal cell lines known to show a rapid retraction of their processes in response to LPA. N1E-115 cells are uniformly sensitive to LPA-induced growth cone collapse (Fig. 4). Expression of PRG-1 in these cells renders the axonal growth cones resistant to LPA-induced collapse (Fig. 4; *15*). Transfection with a control vector solely containing the reporter gene does not alter the LPA-induced responses (Fig. 4). Moreover, analysis of stress fiber formation by phalloidin staining revealed prevention of LPA-induced actin-polymerization (*11*) in PRG-1 overexpressing cells when compared to controls (data not shown; *15*). These data directly demonstrate that PRG-1 expression interferes with LPA-mediated signaling, thus preventing neurite retraction (Fig. 4B).

Sequence alignment analysis revealed that the catalytic domains of lipophosphate phosphatase activity of the LPP family are conserved in PRG-1. These domains catalyze the conversion of phosphatidic acid to diacylglycerol and inorganic phosphate, and are postulated to function both in lipid biosynthesis and in cellular signal transduction (*25, 26*). In order to confirm a phosphatase action of PRG-1, we exchanged the conserved catalytic histidin (His-252) with lysine by site specific mutagenesis (*15*). This exchange has been shown to completely abolish enzymatic function of the catalytic center of LPP-1 (*18*). The same exchange abolished germ cell guiding activity of the Drosophila Wunen protein (*21*). Transfection of this construct into N1E-115 cells no longer prevented LPA-induced retraction of processes as achieved by the wt-construct. These findings provide evidence for the fact that the conserved enzymatic domain present in the LPP-1 family is necessary for attenuating LPA-induced neurite retraction (*18*).

In this study, we describe the identification and functional characterization of a novel lipid phosphate phosphatase, PRG-1, that is specifically expressed in neurons. PRG-1 is upregulated during hippocampal development only after E19 and following ECL, both periods characterized by active axonal outgrowth. Since embryonic neurons, at a time point before their outgrowth from the entorhinal cortex, lack PRG-1 expression, it appears to be the

onset of PRG-1 expression on entorhinal axons that allows them to elongate just after birth and thus penetrate a LPA-rich environment. Moreover, upregulation of PRG-1 expression in regrowing axons following ECL occurs at a post-lesion time point when fibers from the reinnervating associational/commissural tract commence to invade into the denervated entorhinal termination zone (*16, 17, 29*). In addition, the subcellular localization to axons and growth cone-like structures strengthens the concept that PRG-1 plays an important role in axon growth both during development and regenerative sprouting.

LPA is present in the extracellular space (*19, 30*) and is known to act via lysophosphatidate receptors (EDGs), involving intracellular activation of small G-proteins that mediate neurite retraction (*24, 28, 29*). Our *in vitro* studies suggest that PRG-1 antagonizes the activation of EDGs, presumably by reducing the local concentration of active phospholipids surrounding axonal growth cones (see schematic diagram). In fact, a mutant PRG-1 protein lacking a critical residue in the active site was no longer able to protect from LPA-induced neurite retraction. This indicates that PRG-1 expression is a prerequisite for developmental axon growth and regenerative sprouting. This way, PRG-1 is able to regulate activation of lysophosphatidate receptors (EDGs) and thereby modulate axonal outgrowth. Therefore, our data provide evidence for a novel mechanism of axonal outgrowth through the phospholipid-rich environment during development and following brain injury.

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Figure legends:

Figure 1:

PRG-1 mRNA expression is brain-specific.

A. Human PRG-1 amino acid sequence (GenBank acc. #, submitted). The first 300 aa are highly conserved to LPP family members. The other 400 aa (blue boxed sequence) of PRG-1 show no homologies to known sequences. The catalytic histidin (His-252) conserved in all members of the LPP-superfamily is marked with an asterisk. We exchanged this amino acid with lysine to study the functional consequences. **B.** Hydrophobicity profile of the PRG-1 protein predicted by the Kyte and Doolittle algorithms. Numbers in the bottom refer to amino acid residues from the amino terminus. Blue boxed area of PRG-1 is predicted as hydrophilic and located in the cytosol. **C.** Northern blot analysis of PRG-1 mRNA shows a single 5.5 kb band in brain and in testis. **D.** Expression pattern of PRG-1 mRNA in the developing and lesioned rat brain. At embryonic day 16 (E16), no hybridization signal can be detected (right side). Toluidine blue staining of the same section is shown on the left side. On E19, a strong hybridization signal is detectable in the subventricular zone and specifically in the CA1-CA3 principal layer of the hippocampus. Toluidine blue staining of this section is shown on the left side. From the day of birth to adulthood, PRG-1 mRNA is present in all principal layers of the hippocampus and entorhinal cortex. After entorhinal cortex lesion, PRG-1 mRNA is significantly upregulated in all areas of the ipsilateral hippocampus (1 – 5 dal), in the contralateral hippocampus (1 dal) and in the ipsilateral cortex (1 – 5 dal). Statistical differences from respective controls are marked with an asterisk (mean ± S.D.; n = 6), ** $P < 0.001$; two-tailed t test with Bonferroni correction for multiple comparisons. **(E.)** . hi = hippocampus; LV = lateral ventricle; LP = lateral posterior thalamic nucleus; LD = laterodorsal thalamic nucleus; btp = basal telencephalic plate , posterior part; CA1 = cornu ammonis; DG = dentate gyrus; RSG = retrosplenial granular cortex; dal = days after lesion;

Scale bar in E19 equals 850 µm and also applies to E16. Scale bar in P30 equals 740µm and also applies to P0-P15. Scale bar in 1 dal equals 500 µm and also applies to adult.

Figure 2:

PRG-1 is expressed in cellular processes and in hippocampal neurons.

A. Overexpression of PRG-1-eGFP fusion protein (green) in COS-7 show localization in cellular processes. The same subcellular expression pattern can be detected with the anti PRG-1 peptide antibody (red). Colocalization of PRG-1-eGFP and anti PRG-1 shows overlapping in the transfected COS-7 cells and in their processes (marked with white arrows). Scale bar, 10 µm. **B.** Immunocytochemical analysis of PRG-1 in the adult rat hippocampus and after lesion. Pyramidal neurons are labeled in the CA1 and CA3 region. Polymorphic cells are stained in the hilus. Granule cells of the dentate gyrus are also immunopositive. Five days after lesion (dal), a specific increase of immunoreactivity is apparent as an immunopositive band in the outer molecular layer (oml; marked with black arrows). Higher magnification from the boxed area shows immunostained axons (marked with black arrows) and their terminal branches (white arrows). gcl = granule cell layer, hi = hilus. Scale bar equals 580 µm and also applies to adult. Scale bar in 5 dal oml equals 20 µm. **C.** Electron micrograph of a PRG-1 immunopositive axon (delineated by black arrows) with its terminal branch (delineated by red arrows) 5 dal in the oml. Afferent elements including spines are devoid of PRG-1 immunostaining. ax = axon, s = spine. Scale bar equals 0.4 µm.

Figure 3:

Developing entorhinal axons are differentially affected by LPA.

A. Images of representative explants from the rat entorhinal cortex at embryonic day 16 (E 16) and postnatal day 0 (P0) (outgrowing axons marked with white arrows) used to analyze the effect of LPA on neurite retraction. Explants of entorhinal cortex at E16 lack PRG-1

expression, while explants at P0 show high PRG-1 expression levels. The explants were treated with 10 µM LPA or vehicle (0.9% NaCl) for 10 min. Scale bar equals 20 µm. **B.** A scoring system with three different degrees of neurite extensions was used to asses the LPA effect (1: no extensions; 2: short processes; 3: long processes). Experiments were analyzed by three independent observers in a blinded fashion. E16 explants (n = 20) show significant retraction after treatment with LPA in contrast to postnatal explants (n = 22). Statistical differences from controls are marked with an asterisk (mean ± S.D.; explants from three independent set of experiments in total: E16, $n = 36$; P0, $n = 38$) ,*$P < 0.05$; two-tailed t test with Bonferroni correction for multiple comparisons).

Figure 4:

PRG-1 protects from LPA-induced retraction

A. Cell rounding and neurite retraction in response to LPA in N1E-115 cells (wild type = wt). N1E-115 cells were transfected with PRG-1-eGFP fusion construct. For controls, the pEGFP-N1 vector was used. The enzymatic domain of PRG-1 was altered by exchange of the catalytic histidin (aa 252) by lysine (PRG-1 $^{His/Lys}$). Controls transfected with pEGFP-N1 show retraction of neurites and cell rounding after exposure to 10 µM LPA. PRG-1-eGFP transfected cells treated with 10 µM LPA for 10 min show no retraction of processes (marked with white arrows). Transfection with the same vector bearing the His-Lys exchange in the catalytic histidine (PRG-1$^{His/Lys}$) no longer attenuated the LPA induced retraction. Panels on the left show transfected cells, panels in the middle show nuclear staining (Hoechst staining, Roche, Germany), and panels on the right show merged images with f-actin staining. Scale bar equals 20 µm. **B.** A scoring system with three different levels was used to asses neurite outgrowth (1: cell rounding; 2: short processes; 3: long processes). The results from three independent set of experiments (one set with n = 40 for each group) were evaluated by three independent observers in a blinded fashion. Statistical differences from controls are marked

with an asterisk (mean ± S.D.), *P < 0.05; two-tailed t test with Bonferroni correction for multiple comparisons).

Schematic diagram of the proposed axon growth mechanisms in a phospholipid-enriched environment. (Fig. 5)

Axons that are sensitive to a repulsive phospholipid but do not express PRG-1 are unable to cross a phospholipid-rich barrier. In contrast, PRG-1 expressing neurons can grow through a phospholipid-rich zone by locally depleting the extracellular pool of repulsive phospholipids acting as ligands on EDG receptors. This way, PRG-1 may regulate the activation of EDG receptors and thereby modulate axonal outgrowth.

Supporting Online Material

Material and Methods

Animals and surgery

All animals were housed under standard laboratory conditions, and the surgical procedures were performed in agreement with the German law (in congruence with 86/609/EEC) for the use of laboratory animals. All efforts were made to minimize the number of animals used, and all surgical procedures were performed under sufficient anesthesia to minimize animal suffering.

The experimental procedures are described in detail in Bräuer et al., 2001 (S1).

Suppressed Substraction hybridization

We used the SMART cDNA technology from Clontech to generate high yields of full-length, double-stranded cDNA from adult, control and lesioned hippocampus rat RNA. To develop the substraction library, we used the Clontech PCR-Select cDNA Substraction Kit. We used the Clontech PCR-Select differential Screening Kit to analyze the differential expressed cDNAs

***In situ* hybridization and quantification**

For hybridization, we used an antisense oligonucleotide (5'- GCA GAG GTC TGA ATT CTA GTG TCT ATC GTT ATA GTT CCT TAA CAG TGT GGG –3') complementary to bases 425 – 475 of a rat EST cDNA clone (GenBank acc. AW 526088.1). The oligonucleotide was synthesized by Metabion (Munich, Germany). The specificity was confirmed by a BLAST GenBank search to rule out cross-hybridization to other genes.

We used the protocol as described by Bräuer et al., 2001 (S1).

Antibody generation and immunohistochemistry

To design a peptide antibody against PRG-1, we used a sequence in the hydrophilic C-terminal region. The peptide (NH_2-CVGVNGDHHVPGNQ-COOH), representing amino acids 490 – 507 of the PRG-1 rat sequence (GenBank acc. # submitted), was synthesized by BioGenes (Berlin, Germany). The amino-terminal cysteinyl residue, which is not part of the PRG-1 sequence, was included for conjugation of the peptide to a carrier protein. The peptide was conjugated through the cysteinyl sulfhydryl to maleimide activation (keyhole limpet hemocyanin). Rabbits were also immunized by BioGenes. The specificity of the peptide antibody against PRG-1 was further tested on Western-Blot and on brain sections by blocking via peptide incubation prior to adding the antiserum. The protocol for the immunohistochemistry is described in detail by Bräuer et al., 2001 (S2).

Subcellular localization

PRG-1 tagged with the eGFP reporter gene was used to identify the subcellular localization. Golgi apparatus was visualized with the cell tracker BODYPY ceramide (Molecular Probes, Netherlands). The staining protocol was obtained from Molecular Probes.

LPA induced neurite retraction in explants

Entorhinal explants from E16 and P0 rat pups were obtained from timed-pregnant Wistar rats and was cultivated as described (S3). In brief, entorhinal cortex were carefully dissected from the hippocampal anlage and the meninges were removed. Explants were gently transferred with a fire-polished Pasteur pipette into 12-well plates and cultivated on baked glass cover slides coated with laminin and poly-L-lysin (25 µg/ml and 10µg/ml, respectively) in culture medium containing selenium-defined fetal bovine serum [5%] (S4) (Neurobasal medium plus 25 µg/ ml Penecillin/Streptomycin; B-27 supplement). After 24 h, culture medium was exchanged and cultivation was further performed in serum-free Neurobasalmedium for 20 h.

Serum-starved explants were treated with oleoyl-LPA for 10 min and then fixed in 4% paraformaldehyde for 20 min. For F-actin staining, fixed tissues were incubated with FITC-phalloidin (0.1 µg/ml, Sigma, Germany) for 40 min, followed by incubation with HOECHST 33258 dye for 5 min at room temperature. After three washing steps in PBS, explants were coverslipped with ImmuMount (Merck, Germany) prior to analysis. For quantification, a scale score system with three degrees of explant axon outgrowth was used: 1= no extensions; 2 = short processes; 3 = long processes.

Site-directed mutagenesis of PRG-1$^{HIS/LYS}$

The rat PRG-1 full length clone was amplified by Marathon PCR (Clontech, USA) from adult rat hippocampus RNA (GenBank submitted). For transfection studies, the full length PRG-1 coding sequence was fused to EGFP (pEGFP-N1 vector Clontech, USA). The PRG-1$^{His/Lys}$ exchange mutant at the catalytic histidin (His-252) was introduced in the same protein fusion vector by site specific mutagenesis (CAT to AAG).

LPA induced neurite retraction and protection in N1E-115 cells

N1E-115 mouse neuroblastoma cells (ATCC: CRL-2263) were routinely grown in DMEM medium supplemented with selenium-defined fetal bovine serum (10%). The cells were seeded on baked glass cover slides at a density of 10,000 cells/ cm². The next day, cells were transfected with the cationic lipid procedure (FuGene6, Roche, Germany) and cultivated for 24 h. Serum-starvation was performed for 20 h in DMEM medium, followed by treatment with 10 µM oleoyl-LPA or vehicle (0.9% sodium chloride) (*29*). After 10 min, cells were fixed in 4% paraformaldehyde for 20 min at room temperature and analysis was done under an Olympus BX-50 microscope. For quantification, we used a scoring system in accordance with Ebens et al. (1996, S5) with three degrees of explant axon outgrowth:1 = cell rounding; 2 = short processes; 3 = long processes.

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        10          20                                  50          60
MQRAGSSGGR GECDISGAGR LGLEEAARLS CAVHTSPGGG RRPGQAAGMS AKERPKGKVI
        70          80          90          100         110         120
KDSVTLLPCP YPVELPILAS SVVSLYFLEL TDVFKPVHSG FSCYDRSLSM PYIEPTQEAI
       130         140         150         160         170         180
PFLMLLSLAF AGPAITMVG EGILYCCLSK RRNGVGLEPN INAGGCNFNS FLRRAVRFVG
       190         200         210         220         230         240
VHVFGLCSTA LITDIIQLST GYQAPYFLTV CKPNYTSLNV SCKENSYIVE DICSGSDLTV
       250         260         270         280         290         300
INSGRKSFPS QHATLAAFAA VYVSMYFNST LTDSSKLLKP LLVFTFIICG IICGLTRITQ
       310         320         330         340         350         360
YKNHPVDVYC GFLIGGGIAL YLGLYAVGNF LPSDESMFQH RDALRSLTDL NQDPNRLLSA
       370         380         390         400         410         420
KNGSSSDGIA HTEGILNRNH RDASSLTNLK RANADVEIIT PRSPMGKENM VTFSNTLPRA
       430         440         450         460         470         480
NTPSVEDPVR RNASIHASMD SARSKQLLTQ WKNKNESRKL SLQVIEPEPG QSPPRSIEMR
       490         500         510         520         530         540
SSSEPSRVGV NGDHHGPGNQ YLKIQPGAVP GCNNSMPGGP RVSIQSRPGS SQLVHIPEET
       550         560         570         580         590         600
QENISTSPKS SSARAKWLKA ARKTVACNRS NSQPRIMQVI AMSKQQGVLQ SSPKNTEGST
       610         620         630         640         650         660
VSCTGSIRYK TLTDHEPSGI VRVEAHPENN RPIIQIPSTE GEGSGSWKWK APEKGSLRQT
       670         680         690         700         710         720
YELNDLNRDS ESCESLKDSF GSGDRKRSNI DSNEHHHHGI TTIRVTPVEG SEIGSETLSI
       730         740         750         760         770         780
SSSRDSTLRR KGNIILIPER SNSPENTRNI FYKGTSPTRA YKD..... ..........
```

Human PRG1
SEQ ID NO: 1

```
        10         20         30         40         50         60
MQRAGSSGAR GECDISGAGR LRLEQAARLG GRTVHTSPGG GLGARQAAGM SAKERPKGKV
        70         80         90        100        110        120
IKDSVTLLPC FYFVELPILA SSVVSLYFLE LTDVFKPVES GFSCYDRSLS MPYIEPTQEA
       130        140        150        160        170        180
IPFLMLLSLA FAGPAITIMV GEGILYCCLS KRRNGAGLEP NINAGGCNFN SFLRRAVRFV
       190        200        210        220        230        240
GVHVFGLCST ALITDIIQLS TGYQAPYFLT VCKPNYTSLN VSCKENSYIV EDICSGSDLT
       250        260        270        280        290        300
VINSGRKSFP SQHATLAAFA AVYVSMYFNS TLTDSSKLLK PLLVFTPIIC GIICGLTRIT
       310        320        330        340        350        360
QYKNHPVDVY CGFLIGGGIA LYLGLYAVGN FLPSEDSMLQ HRDALRSLTD LNQDPSRVLS
       370        380        390        400        410        420
AKNGSSGDGI AHTEGILNRN HRDASSLTNL KRANADVEII TPRSPMGKES MVTFSNTLPR
       430        440        450        460        470        480
ANTPSVEDPV RRNASIHASM DSARSKQLLT QWKSKNESRK MSLQVMDTEP EGQSPPRSIE
       490        500        510        520        530        540
MRSSSEPSRV GVNGDHHVPG NQYLKIQPGT VPGCNNSMPG GPRVSIQSRP GSSQLVHIPE
       550        560        570        580        590        600
ETQENISTSP KSSSARAKWL KAAEKTVDCN RSNNQPRIMQ VIAMSKQQGV LQSSPKNAEG
       610        620        630        640        650        660
STVTCTGSIR YKTLTDHEPS GIVRVEAHPE NNRPIIQIPS STEGEGSGSW KWKVPEKSSL
       670        680        690        700        710        720
RQTYELNDLN RDSESCESLK DSFGSGDRKR SNIDSNEHHH HGITTIRVTP VEGSEIGSET
       730        740        750        760        770        780
LSVSSSRDST LRRKGNIILI PERSNSPENT RNIFYKGTSP TRAYKD.... ..........
```

Mouse PRG1

SEQ ID NO:2

```
      10         20         --         50         60
MISTKEKNKI PKDSMTLLPC FYFVELPIVA SSIVSLYFLE LTDLFKPAKV GFQCYDRTLS
      70         80         90        100        110        120
MPYVETNEEL IPLLMLLSLA FAAPAASIMV AEGMLYCLQS RLWGRAGGPA GAEGSINAGG
     130        140        150        160        170        180
CNFNSFLRRT VRFVGVHVPG LCATALVTDV IQLATGYHTP FFLTVCKPNY TLLGTSCEVN
     190        200        210        220        230        240
PYITQDICSG HDIHAILSAR KTFPSQHATL SAFAAVYVSV SPAPHCPSQA LLLTRGEPSL
     250        260        270        280        290        300
TPTPMPQMYF NSVISDTTKL LKPILVFAFA IAAGVCGLTQ ITQYRSHPVD VYAGFLIGAG
     310        320        330        340        350        360
IAAYLACHAV GNFQAPPAEK PAAPAPAKDA LRALTQRGHD SVYQQNKSVS TDELGPPGRL
     370        380        390        400        410        420
EGAPRPVARE KTSLGSLKRA SVDVDLLAPR SPMAKENMVT FSHTLPRASA PSLDDPARRH
     430        440        450        460        470        480
MTIHVPLDAS RSKQLISEWK QKSLEGRGLG LPDDASPGHL RAPAEPMAEE EEEEEDEEEE
     490        500        510        520        530        540
EEEEEEEDEG PAPPSLYPTV QARPGLGPRV ILPPRAGPPP LVHIPEEGAQ TGAGLSPKSG
     550        560        570        580        590        600
AGVRAKWLMM AEKSGAAVAN PPRLLQVIAM SKAPGAPGPK AAETASSSSA SSDSSQYRSP
     610        620        630        640        650        660
SDRDSASIVT IDAHAPHHPV VHLSAGGAPW EWKAAGGGAK AEADGGYELG DLARGFRGGA
     670        680        690        700        710        720
KPPGVSPGSS VSDVDQEEPR PGAVATVNLA TGEGLPPLGA ADGALGPGSR ESTLRRHAGG
     730        740        750        760        770        780
LGLAEREAEA EAEGYFRKMQ ARRPPD.... .......... ....................
```

Human PRG2

SEQ ID NO:3

```
        10          20          --                  50          60
MLAMKEKNKT PKDSMTLLPC FYFVELPIVA SSIVSLYFLR LTDLFKPAKV GFQCYDRALS
        70          80          90          100         110         120
MPYVETNEEL IPLLMLLSLA FAAPAASIMV GEGMVYCLQS RLWGRGPGGV EGSINAGGCN
       130         140         150         160         170         180
FNSFLRRTVR FVGVHVFGLC ATALVTDVIQ LATGYHTPFF LTVCKPNYTL LGTSCESNPY
       190         200         210         220         230         240
ITQDICSGHD THAILSARKT FPSQHATLSA FAAVYVSMYF NAVISDTTKL LKPILVFAFA
       250         260         270         280         290         300
IAAGVCGLTQ ITQYRSHFVD VYAGFLIGAG IAAYLACHAV GNFQAPPAEK VPTPAPAKDA
       310         320         330         340         350         360
LRALTQRGHE SMYQQNKSVS TDELGPPGRL EGVPRPVARE KTSLGSLKRA SVDVDLLAPR
       370         380         390         400         410         420
SPMGKEGMVT FSNTLPRVST PSLDDPARRH MTIHVPLDAS RSRQLIGEWK QKSLEGRGLG
       430         440         450         460         470         480
LPDEASPVHL RAPAEQVAEE EEEEEEEEER EEEEEEEEGP VPPSLYPTVQ ARPGLGPRVI
       490         500         510         520         530         540
LPPRPGPQPL VHIPEEGVQA GAGLSPKSSS SSVRAKWLSV AEKGGGPVAV APSQPRVANP
       550         560         570         580         590         600
PRLLQVIAMS KAAGGPKAET ASSSSASSDS SQYRSPSDRD SASIVTIDAH APHHPVVHLS
       610         620         630         640         650         660
AGSTPWEWKA KVVEGEGSYE LGDLARGFRS SCKQPGMGPG SPVSDVDQEE PRFGAVATVN
       670         680         690         700         710         720
LATGEGLPPP GASEGALGAG SRESTLRRQV GGLAEREVEA EAESYYRRMQ ARRYQD*...
```

Mouse

PRG2

SEQ ID NO: 4

```
        10         20                                  50         60
GGATCCACTA GTAACGGCCG CCAGTGTGCT GGAATTCGCC CTTGAAGCCA TTGCAGCAAC
        70         80         90        100        110        120
AGCTTGGAGG AGGGAGCTGG ACGTCGTCTC TCGCCAGAAA AACGGGGAGC AGGAGCCAGA
       130        140        150        160        170        180
CTAAAGGAGG AAGAGGACTG GCCCGCTCAG GGAATAGCTG GGTTGCTGCA AAaAgggGCg
       190        200        210        220        230        240
GggAGAaGGC GGGGGCGCTG CATGCAGCgC GCTGGCTCCA GCGGTGGCCG CGGGGAATGT
       250        260        270        280        290        300
GACATCAGCG GCGCCGGGCG CTTGGGGCTG GAGGAGGCGG CTCGCCTCAG CTGCGCTGTG
       310        320        330        340        350        360
CACACCTCGC CCGGGGGAGG ACGCAGACCC GGGCAGGCGG CAGGGatgtc ggcgaaggag
       370        380        390        400        410        420
aggccaaagg gcaaagtgat caaggacagc gtcacccctcc tgccctgctt ttatttcgtc
       430        440        450        460        470        480
gagttgccta tattggcatc atcggtggtt agcctctatt tcctcgaact cacagatgtc
       490        500        510        520        530        540
ttcaaacctg tgcactctgg atttagctgc tatgaccgga gtcttagcat gccgtacatt
       550        560        570        580        590        600
gaaccaaccc aggaggcaat tccattcctc atgttgctta gcttggcttt tgctggacct
       610        620        630        640        650        660
gcaattacga ttatggtagg agaaggaatt ctctactgtt gcctctccaa aagaagaaat
       670        680        690        700        710        720
ggggtcggac tagagcccaa cattaatgct ggaggctgca acttcaattc cttcctcaga
       730        740        750        760        770        780
cgagctgtca gattcgttgg tgttcatgta tttggattat gctctacagc tctcattaca
       790        800        810        820        830        840
gatatcatac agctgtccac aggatatcaa gcaccttact ttctgactgt gtgcaaacca
       850        860        870        880        890        900
aactatacct ctctgaatgt atcttgcaaa gaaaattcct acattgtgga agatatttgc
       910        920        930        940        950        960
tcaggatctg acctcacagt tatcaacagt ggcagaaagt ccttcccttc tcaacatgca
       970        980        990       1000       1010       1020
acccttgctg cctttgcagc tgtgtatgtt tcgatgtact tcaattccac attaacggat
      1030       1040       1050       1060       1070       1080
tcctctaagc ttctgaaacc tctcttggtc ttcacattta tcatctgtgg aataatctgc
      1090       1100       1110       1120       1130       1140
gggctaacac ggataacttca gtataagaac cacccagttg atgtctattg tggcttttta
      1150       1160       1170       1180       1190       1200
ataggaggag gaattgcact gtacttgggc ttgtatgctg tggggaattt cctgcccagt
      1210       1220       1230       1240       1250       1260
gatgagagta tgtttcagca cagagacgcc ctcaggtctc tgacagacct caatcaagat
      1270       1280       1290       1300       1310       1320
cccaaccgac ttttatctgc taaaaatggt agcagcagtg atggaattgc tcatacagaa
      1330       1340       1350       1360       1370       1380
ggcatcctca accgaaacca cagagatgct agctctctga caaatctcaa aagagcaaat
      1390       1400       1410       1420       1430       1440
gctgatgtgg aaatcattac tccacggagc cccatgggga aggagaacat ggttaccttc
      1450       1460       1470       1480       1490       1500
agcaatacct tgccgcgagc caatacccca tctgtagaag accctgtcag aagaaatgcg
      1510       1520       1530       1540       1550       1560
agcattcatg cctctatgga ttccgctcga tcaaagcagc tcctcaccca gtggaagaat
      1570       1580       1590       1600       1610       1620
aagaatgaaa gtcgaaagtt gtccttgcaa gttatagagc ctgagcctgg gcagtcacca
      1630       1640       1650       1660       1670       1680
cccagatcca tagaaatgag gtcaagctca gagccatcga gggtaggggt gaatggagac
```

SEQ ID NO:5

human PRG1

START bp 202

STOP bp 2490

SEQ ID NO:5

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      1690        1700                                    1730        1740
  caccatggtc ctggcaatca gtacctcaaa atccagcctg gcgctgtccc cggatgtaac
      1750        1760        1770        1780        1790        1800
  aacagcatgc ctggagggcc aagagtgtcc attcagtccc gtcctgggtc ctcacagttg
      1810        1820        1830        1840        1850        1860
  gtgcacatcc ctgaggagac tcaggaaaac ataagcacat cccccaaaag cagctctgct
      1870        1880        1890        1900        1910        1920
  cgggccaagt ggttaaaagc tgctgaaaag actgtggcct gtaacagaag caacagccag
      1930        1940        1950        1960        1970        1980
  ccccgaatca tgcaagtcat agccatgtcc aagcagcagg gtgtcctcca aagcagcccc
      1990        2000        2010        2020        2030        2040
  aagaacactg aaggcagcac ggtctcctgc actggctcca tccgctataa aaccttgaca
      2050        2060        2070        2080        2090        2100
  gaccatgagc ccagtgggat agtgagggtt gaggctcacc cagagaacaa caggccccatc
      2110        2120        2130        2140        2150        2160
  atacagatcc cgtccactga aggtgaaggc agtggctcct ggaagtggaa agccctgaa
      2170        2180        2190        2200        2210        2220
  aagggcagcc ttcgccaaac ttacgagctc aacgatctca acagggactc agaaagctgt
      2230        2240        2250        2260        2270        2280
  gagtctctga aagacagcct tggttctgga gatcgcaaga gaagcaacat tgatagcaat
      2290        2300        2310        2320        2330        2340
  gagcatcacc accacggaat taccaccatc cgcgtcaccc cagtagaggg cagcgaaatt
      2350        2360        2370        2380        2390        2400
  ggctcagaga cgctgtccat ttctttcttcc cgcgactcca ccctgcggag aaagggcaat
      2410        2420        2430        2440        2450        2460
  atcattctaa tccctgaaag aagcaacagc ccggaaaaca ctagaaatat cttctacaaa
      2470        2480        2490        2500        2510        2520
  ggaacctccc ccacacgggc ttataaggat tgagtgatgt ccattccatc attagggcta
      2530        2540        2550        2560        2570        2580
  ctcgcaaaag accatatgtt gattctacct gtgttctgtt ccagcgaatt gggaagtctc
      2590        2600        2610        2620        2630        2640
  accaagctag attgtctacc atcagcccag aactctgtaa cttttcagaa ctgctatact
      2650        2660        2670        2680        2690        2700
  caaacttgca gatctcacat caaggagagg gaaaagcaca atgcaagaac ctaactaacg
      2710        2720        2730        2740        2750        2760
  tgatgatatg aagagttttc ttaagacctg tcgtcaaact taaaaggttt tgcagagggc
      2770        2780        2790        2800        2810        2820
  agtatcaaaa gaaagtggtt ttcttcaaat gtatactatt ttacttcctg aatgtgccaa
      2830        2840        2850        2860        2870        2880
  ctttggggat ttttcttttat agtgagctgt gggaacccag aacacacacg ttttccctac
      2890        2900        2910        2920        2930        2940
  agcagaggcc atgcagtatt atatattcat tttgcagaat ctgcacctac agctcaatac
      2950        2960        2970        2980        2990        3000
  gggtggtgct gattattata gtacatatac catgtaaact ctcaaactct atttagctgt
      3010        3020        3030        3040        3050        3060
  gaaatagtgg tgtgcaattc cttgttaaag aaatgctact ttattaagaa gatgctggct
      3070        3080        3090        3100        3110        3120
  gctttgtgtt agaataggac acccccgcagc ttctctgtag tggctctgtc acagtcaaaa
      3130        3140        3150        3160        3170        3180
  aatgaaaagg tttttgtgcg tttcttcaaa attctgcttt cttcaacatc aaaaattgtg
      3190        3200        3210        3220        3230        3240
  tagaaatatt ttcagtgaaa gggaataact agtacttttc tgcatagttt ttcttctgct
      3250        3260        3270        3280        3290        3300
  tacttttttat ttaagtatag gtactgctaa tgaatctgtt ttcttagtga gtaaattttgc
      3310        3320        3330        3340        3350        3360
  ataatttttat aaatattatt ttagagaatc ttttgaaatt gttgtgatca tatttttgctt
```

SEQ ID NO:5
continuation 1

SEQ ID NO
continuation 1

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      3370       3380                                  3410       3420
 tctatggctt ctccttaact tattgattaa ttttttgaag ttatagatat gttctcctat
      3430       3440       3450       3460       3470       3480
 tttaaaagca aaaataacaa ttgacattcc ttgagcaaaa tatactgctg tgaatttgca
      3490       3500       3510       3520       3530       3540
 aacaagaaat ctgagccaaa acttgacatt gtgggttaca ttgccagaaa tgttggtcaa
      3550       3560       3570       3580       3590       3600
 gtttgccctt agatgtctac aactagctgg cataggttgc catcttaaca agtaatctaa
      3610       3620       3630       3640       3650       3660
 aagtcccatt cggttctaca ttattaactt ttttttttta tatcctgatg accagtaaat
      3670       3680       3690       3700       3710       3720
 tagagccaca ctggttaagt ttgactcgtc tctaaaacgt ttttgttaat tggacaccaa
      3730       3740       3750       3760       3770       3780
 gaggaagaat ctgaaaaaaa aatgcatgtt ggtaagtaaa agtatcttcac ggtacaaatt
      3790       3800       3810       3820       3830       3840
 aagaatgact ttcttcaaaa tatctgaata ggtgcagttt tagtttaaca tgcaaacaac
      3850       3860       3870       3880       3890       3900
 cattgttgct acctatcctg aatcaagcct tgagcctaaa tcaaagcaaa ccaataccat
      3910       3920       3930       3940       3950       3960
 tgataagaag aagataaaaa caaaatattt tggagtgttt tccaacttaa agtatgaaga
      3970       3980       3990       4000       4010       4020
 catactcagt tcttggaact tagtattaaa cctttttttat gccatttcat aagaattccg
      4030       4040       4050       4060       4070       4080
 atatatactt gatgattgcc aaggggatga aaggaaacaa cagagatggt tgatctgatc
      4090       4100       4110       4120       4130       4140
 ttagctcact ttccaataac agaaggagtt gtttacagat gaatagtatc acatcattat
      4150       4160       4170       4180       4190       4200
 caatttccac atgaaaaagg tggagctttc tagaaaaacc aacctctaag gcattaggaa
      4210       4220       4230       4240       4250       4260
 tttagctgaa accagcagaa ttgaaaactc tggcaataaa acatggactc aaccatatcc
      4270       4280       4290       4300       4310       4320
 cttctggcaa tttccttctc agagagggga gtgggaataa aatgttgcct tccccacttc
      4330       4340       4350       4360       4370       4380
 tcaccaccac cgccatcatg acgctcatac tggcttttgc ctgtttgtag aggaaaaggt
      4390       4400       4410       4420       4430       4440
 gggctggttt tagtacttctg aaggacaaaa acaagcaaac aaaaaccccct gctgcagcat
      4450       4460       4470       4480       4490       4500
 ttcaggtgca gtatgatatt tcctaatctt tcctattttct taacaaaaga ttttaaagta
      4510       4520       4530       4540       4550       4560
 cttctctagt cattgaagtt ttttttttctt tacataaata ttgatatatt ctttttctac
      4570       4580       4590       4600       4610       4620
 tcaaagtgcc aaaggctaca gtttttaatg acttaacaaa ttgtaccaca ttgttaagga
      4630       4640       4650       4660       4670       4680
 catataatga tagacactag aactcagacc tctgcatgta tatttgataa catgtctttt
      4690       4700       4710       4720       4730       4740
 gtaaaacaaa aattacaaaa aaatttgttt acattccact ggtaccttaa tttaaaataa
      4750       4760       4770       4780       4790       4800
 atcagactaa aaggtggtat ctcttcttag tgttctattt atcttatttg ctaatgggag
      4810       4820       4830       4840       4850       4860
 cacttcttcc tttgttaggc tgtgctttac tgataaaacc aagtattgaa taaagagagt
      4870       4880       4890       4900       4910       4920
 taattatctt tttaaagtAA ATAAAATTAT GAAAATATAT ATAGTATATA TAAAGTCCTG
      4930       4940       4950       4960       4970       4980
 TGTTTAAAAA AATGTTATGC AATGTTTTCC AAACTGATAA AGTTTGTAAA GTGCTATAAA
      4990       5000       5010       5020       5030       5040
 TGTATTTTGT TAAGtaCAGA TAAAAGCTAT TGTGTGAGTA TATTGTGCTA AAATCATAGA
```

SEQ ID NO:5

continuation 2

SEQ ID NO:5
continuation 2

5050 5060 5090 5100

AATAAAGATT AGATTTCTTC ATCAAAAAAA AAAAAAAAAA AAA.....

SEQ ID NO: 5

continuation 3

human PRG2

START bp 64

STOP bp 2304

SEQ ID NO:

```
        10         20                                  50         60
aggagacgcg ctttgtgctg ggcgccggcc gcgccagcca cggcctgcgg cgcccgcggc
        70         80         90        100        110        120
accatgatct ccaccaagga gaagaacaag atcccgaagg acagcatgac gcttctgccc
       130        140        150        160        170        180
tgcttctact tcgtggagct gcccatagtg gcttcttcca tcgtatcctt gtacttcctg
       190        200        210        220        230        240
gagctgaccg acctcttcaa gccggccaag gtgggcttcc agtgctatga ccgcactctc
       250        260        270        280        290        300
tccatgccct acgtggagac caacgaggag ctcatcccgc tgctgatgct gctcagcttg
       310        320        330        340        350        360
gccttcgcgg ccctgccgc ctcgatcatg gtggccgagg gcatgttgta ctgtctgcag
       370        380        390        400        410        420
tcccggctgt ggggccgtgc cgggggccc gccggggcgg agggcagcat caacgccggc
       430        440        450        460        470        480
ggctgcaact tcaactcctt cctgcggcgt acggtgcggt ttgtgggtgt ccacgtgttc
       490        500        510        520        530        540
ggcctgtgtg ccacagccct ggtgacggac gtgatccagc tggccacggg ttaccacact
       550        560        570        580        590        600
ccctttttcc tcaccgtctg caagcccaac tacactctcc tgggcacgtc ctgcgaggtc
       610        620        630        640        650        660
aacccctaca tcacgcagga catctgctcc ggccacgaca tccacgccat cctgtctgca
       670        680        690        700        710        720
cggaagacct tcccgtccca gcacgccacg ctgtcagcct tcgccgcggt ctatgtgtcg
       730        740        750        760        770        780
gtgagtccgg cacctcactg ccctttccag gccctcttgc tgaccgtgg ggagccctcc
       790        800        810        820        830        840
ctgaccccaa ccccatgcc ccagatgtac ttcaactcgg tcatctcgga caccaccaag
       850        860        870        880        890        900
ctgctgaagc ccatcctggt cttcgcgttt gccatcgccg cgggcgtatg cgggctcacg
       910        920        930        940        950        960
cagatcacgc agtaccgcag ccaccctgtg gacgtgtatg ccggcttcct catcggggcg
       970        980        990       1000       1010       1020
ggcatcgctg cctacctggc ctgccacgcg gtgggcaact tccaggcccc acctgcagag
      1030       1040       1050       1060       1070       1080
aagcccgcgg cccaggcccc cgccaaggac gcgctgcggg ccctgacgca gcggggccac
      1090       1100       1110       1120       1130       1140
gactcggttt atcagcagaa taagtcggtg agcaccgacg agctggggcc cccagggcgg
      1150       1160       1170       1180       1190       1200
ctggagggcg cgcccccggcc cgtggcccgc gagaagacct cgctgggcag cctgaagcgc
      1210       1220       1230       1240       1250       1260
gccagcgtgg acgtggacct gctggccccg cgcagccccca tggccaagga gaacatggtg
      1270       1280       1290       1300       1310       1320
accttcagcc acacgctgcc cagggccagc gcgccctcgc tggacgaccc cgcgcgccgc
      1330       1340       1350       1360       1370       1380
cacatgacca tccacgtgcc gctggacgcc tcgcgctcca agcagctcat cagcgagtgg
      1390       1400       1410       1420       1430       1440
aagcagaaga gcctggaggg ccgcggcctg gggctgcccg acgacgccag ccccgggcac
      1450       1460       1470       1480       1490       1500
ctgcgcgcgc ccgccgaacc catggcggag gaggaggaag aggaggagga cgaagaggaa
      1510       1520       1530       1540       1550       1560
gaggaggagg aggaagagga ggaggacgag ggcccggccc cgccctcgct ctaccccacc
      1570       1580       1590       1600       1610       1620
gtgcaggcgc ggccggggct ggggcctcgg gtcatcctcc caccgcgcgc ggggccgccg
      1630       1640       1650       1660       1670       1680
ccgctggtgc acatccccgga ggagggcgcg cagacggggg ccggcctgtc ccccaaaagc
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SEQ ID NO: 6

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      1690        1700                                    1730        1740
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      1750        1760        1770        1780        1790        1800
aaccctccgc ggctgctgca ggtcatcgcc atgtccaagg ctccgggcgc gccgggcccc
      1810        1820        1830        1840        1850        1860
aaggcggccg agacggcgtc gtcgtccagc gccagctccg actcctcgca gtaccggtcg
      1870        1880        1890        1900        1910        1920
ccgtcggacc gcgactccgc cagcatcgtg accatcgacg cgcacgcgcc gcaccacccc
      1930        1940        1950        1960        1970        1980
gtggtgcacc tgtcggccgg cggcgcgccc tgggagtgga aggcggcggg cggcgggccc
      1990        2000        2010        2020        2030        2040
aaggcggagg ccgacggcgg ctacgagctg ggggacctgg cgcgcggctt ccgcggcggg
      2050        2060        2070        2080        2090        2100
gccaagcccc cgggcgtgtc ccccggctcg tcggtcagcg acgtggacca ggaggagccg
      2110        2120        2130        2140        2150        2160
cggttcgggg ccgtggccac cgtcaacctg gccacgggcg aggggctgcc cccgctgggc
      2170        2180        2190        2200        2210        2220
gcggccgatg gggcgctggg cccgggcagc cgggagtcca cgctgcggcg ccacgcgggc
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ggcctggggc tggcggagcg cgaggcggag gcggaggccg agggctactt ccgcaagatg
      2290        2300        2310        2320        2330        2340
caggcgcgcc gcttcccccga ctagcgcggc ggggccgggg gcgggcgggg ggcgggccga
      2350        2360        2370        2380        2390        2400
gggcgcgggc ggccgc.... .......... .......... .......... ..........
```

SEQ ID NO: 6 Continuation

Claims

1. An isolated polypeptide comprising the same or substantially the same amino acid sequence selected from the group consisting of SEQ ID NO:1-4, or a splice variant or a salt thereof.

2. A partial peptide of the protein according to claim 1, or a salt thereof.

3. A DNA which comprises a DNA encoding the protein according to claim 1 and/or 2.

4. A DNA according to claim 3 selected from the group consisting of SEQ ID NO:5-6.

5. A recombinant vector which comprises the DNA according to claim 3.

6. A transformant transformed with the recombinant vector according to claim 5.

7. A method of producing the protein or its salt according to claim 1, which comprises culturing the transformant according to claim 6, and producing an accumulating the protein according to claim 1.

8. An antibody to the protein according to claim1, the partial peptide according to claim 2, or a salt thereof.

9. A method of determining a ligand to the protein or its salt according to claim 1, which comprises using the protein according to claim 1 or the partial peptide according to claim 2, or a salt thereof.

10. A pharmaceutical composition comprising a polypeptide and/or DNA according to one of the claims 1-5.

Abstract

Outgrowth of axons in the central nervous system is governed by specific molecular cues. Molecules detected so far act as ligands that bind to specific receptors. Here, we report on a novel membrane-associated lipid phosphate phosphatase we named plasticity-related gene-1 (PRG-1), which facilitates axonal outgrowth during development and regenerative sprouting. PRG-1 is specifically expressed in neurons and is located in the membranes of outgrowing axons. There, it acts as an ecto-enzyme and attenuates phospholipid-induced axon collapse in neurons and outgrowth in the hippocampus. Thus, we unraveled here a novel mechanism by which axons are able to control phospholipid-mediated signaling and overcome the growth-inhibiting phospholipid-rich environment of the extracellular space.

Fig. 1

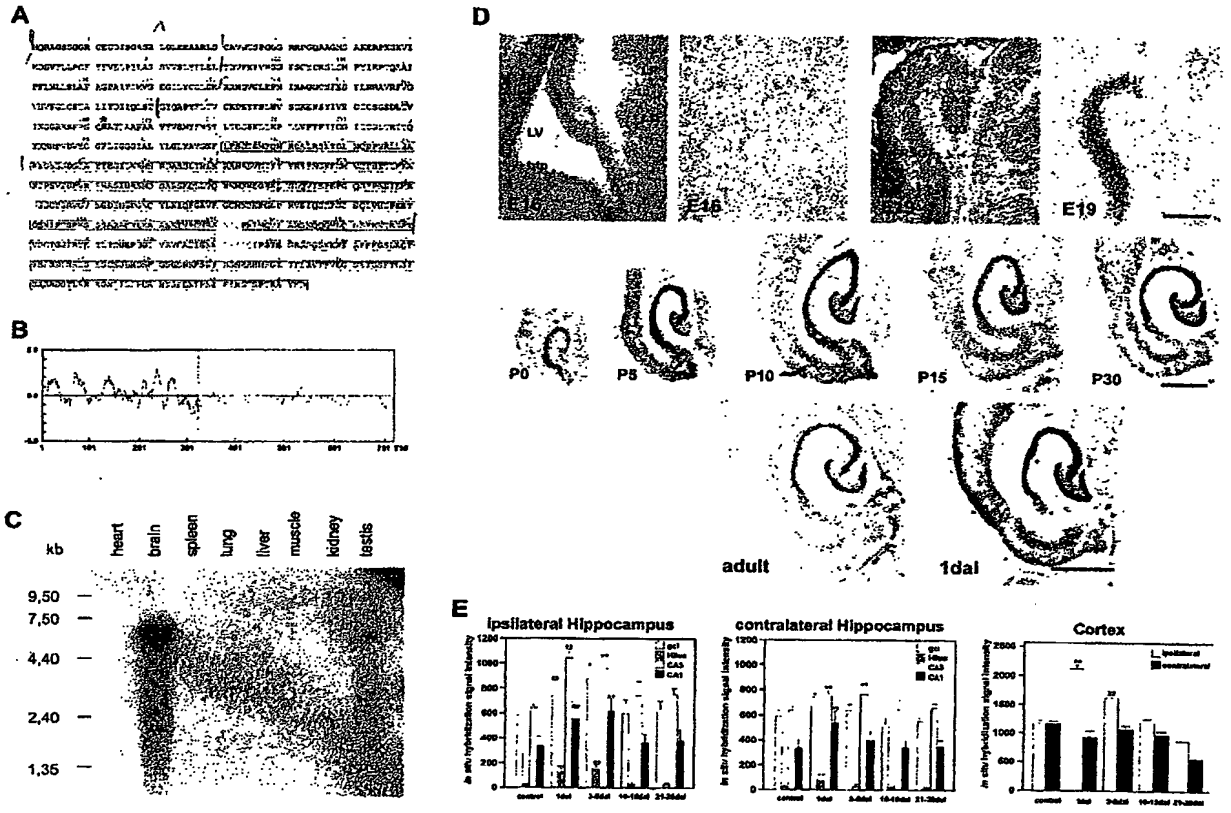


Fig. 2

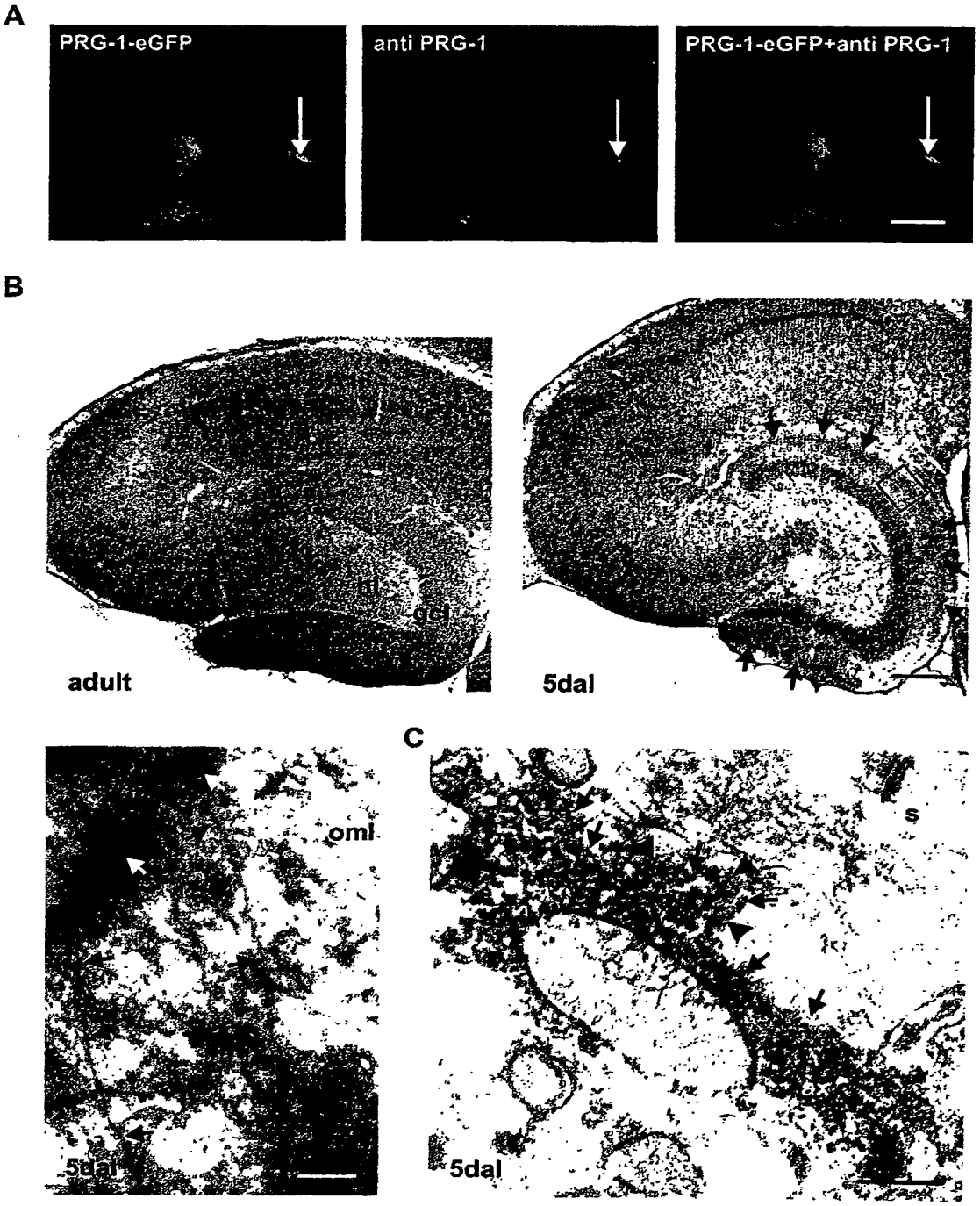

A
PRG-1-eGFP
anti PRG-1
PRG-1-eGFP+anti PRG-1
B
gcl
adult
5dal
C
oml
5dal
s
5dal

Fig. 3

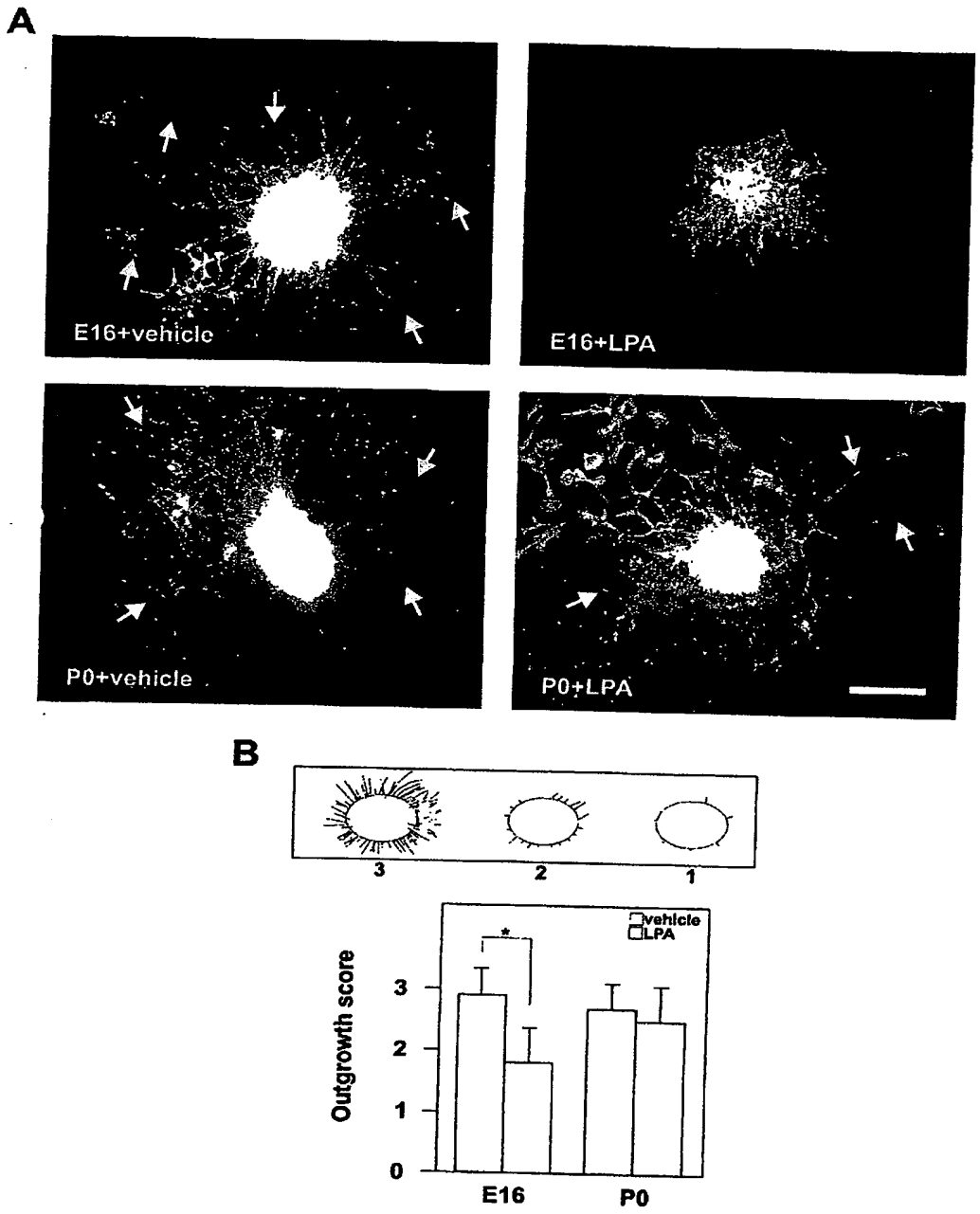
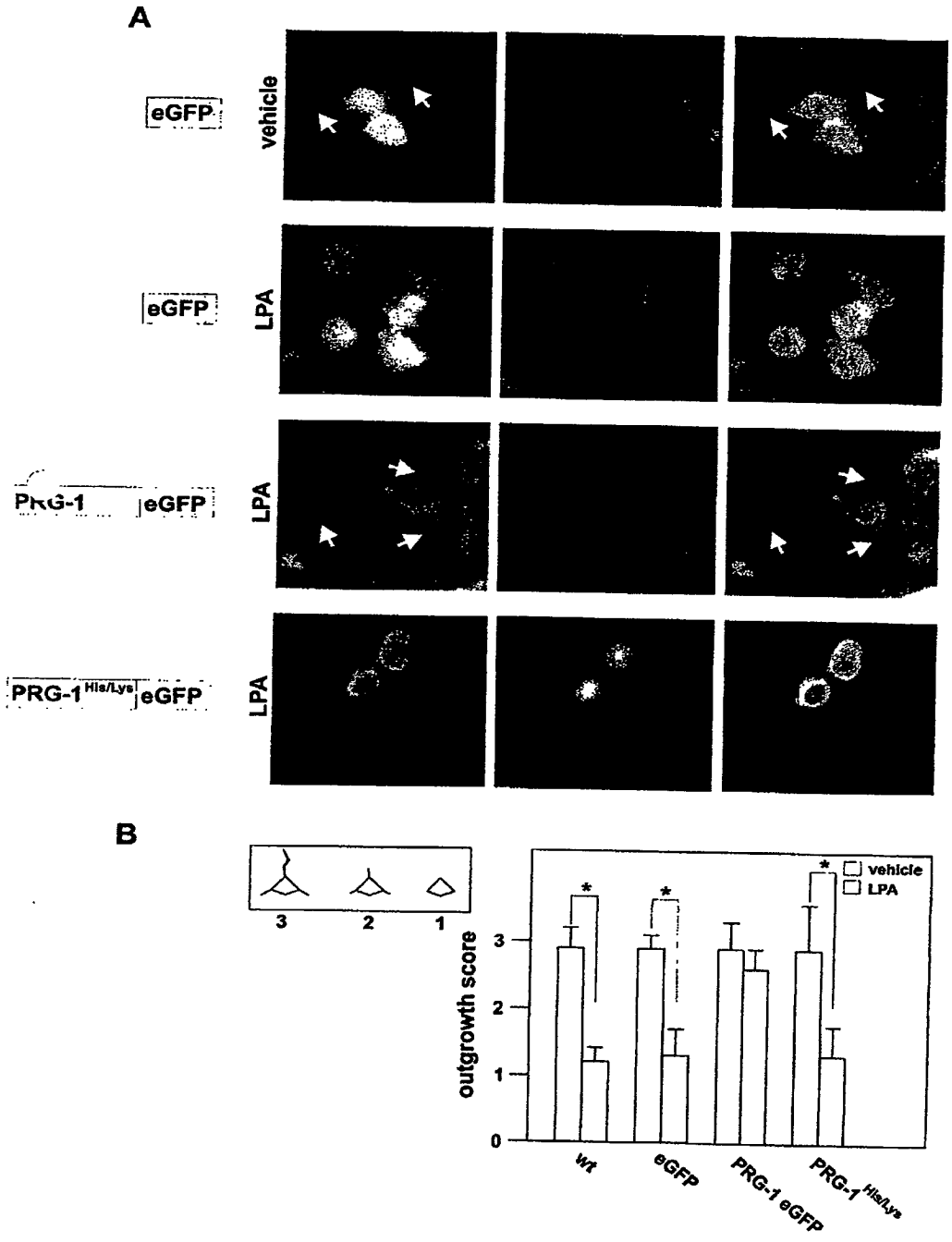

A
E16+vehicle
E16+LPA
P0+vehicle
P0+LPA
B
3
2
1
vehicle
LPA
*
Outgrowth score
0
1
2
3
E16
P0

Fig. 4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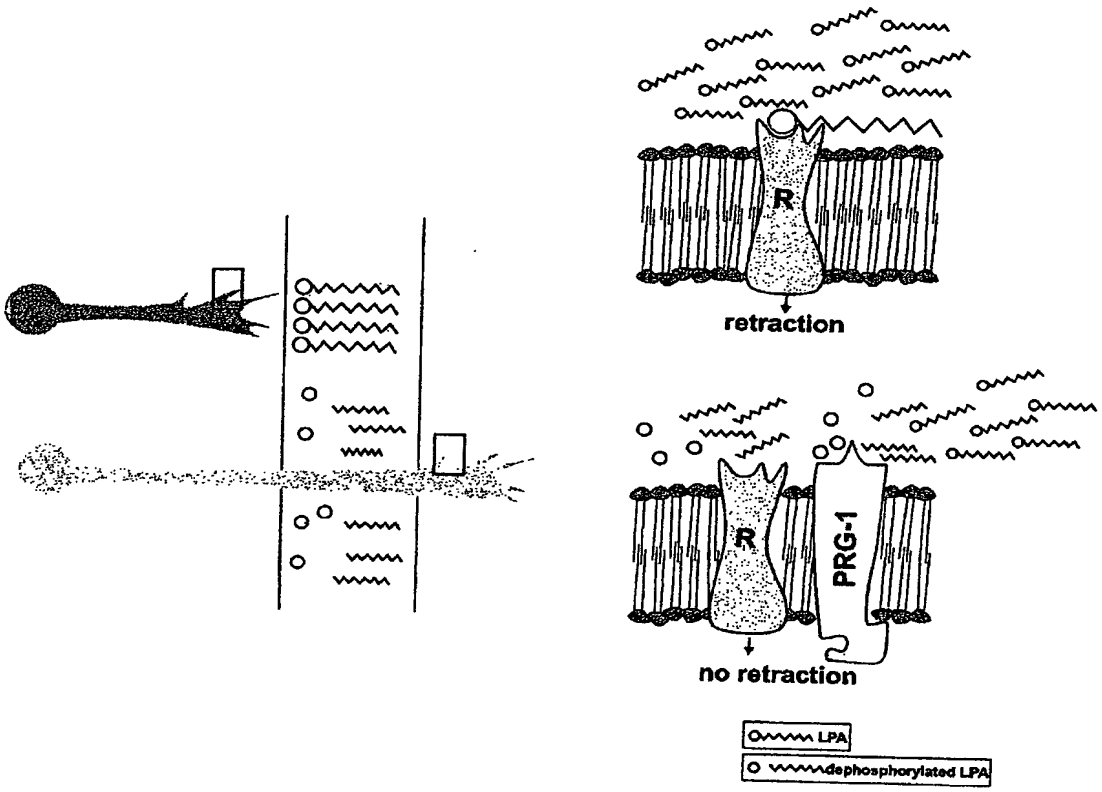
eGFP
vehicle
eGFP
LPA
PRG-1 eGFP
LPA
PRG-1 His/Lys eGFP
LPA
B
3
2
1
outgrowth score
0
1
2
3
vehicle
LPA
*
*
*
wt
eGFP
PRG-1 eGFP
PRG-1 His/Lys

Fig. 5



SCHEMATIC DIAGRAM